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Alterations in human lymphocyte DNA caused by sulfur mustard can be mitigated by selective inhibitors of poly(ADP-ribose) polymerase[1]

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Abstract

Changes in genomic DNA caused by exposure to the cytotoxic alkylating agent, 2,2′-dichlorodiethyl sulfide (sulfur mustard; HD), alone or in combination with selective inhibitors of poly(ADP-ribose) polymerase (PARP), were analyzed as a function of HD concentration and post-exposure time. Preparations of human peripheral blood lymphocytes were exposed to HD (1×10^{-8} M–1×10^{-3} M), and incubated at 37°C for 0–24 h. Total genomic DNA was extracted from these cells and compared with DNA from control cells of the same donor using agarose gel electrophoresis. The effects of HD on genomic DNA depended on the HD concentration and the length of the post-exposure time interval. DNA fragmentation was detected as early as 2 h after exposure to 3×10^{-4} M HD, or at 24 h after exposure to 6×10^{-6} M HD. The qualitative DNA pattern, as well as the extent of DNA fragmentation, changed with post-exposure time. Exposure to HD caused a time-dependent shift in the DNA cleavage pattern from an oligonucleosome-sized 'DNA ladder' characteristic of apoptotic cell death, to a 'broad band' pattern characteristic of necrotic cell death. DNA fragmentation was not observed if cells were killed with heat or with Lewisite. Treatment of cells with selective PARP inhibitors consistently altered the DNA fragmentation caused by HD exposure. The inhibitors arrested DNA fragmentation at the DNA ladder stage. This effect only was observed if the PARP inhibitors were applied within 8 h of HD exposure. We conclude that early inhibition of PARP activity can induce a switch in the mechanism of cell death caused by HD. Such a switch may be useful therapeutically to convert a lytic, pro-inflammatory cell death that includes the disintegration of dying cells (necrosis), into a slower, programmed cell death that includes absorption of dying cells (apoptosis). 0167-4889/98/$ – see front matter © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Sulfur mustard; DNA fragmentation; Apoptosis; Necrosis; Lymphocyte; Poly(ADP-ribose) polymerase

1. Introduction

The alkylating agent, 2,2′-dichlorodiethyl sulfide (sulfur mustard; HD), is a potent vesicant, or 'blister agent' that causes severe lesions of exposed skin, lungs, or eyes (reviewed in [1]). There is, to date, no effective antidote. Herriott and Price implicated DNA as an important target in the cellular effects of HD during the late 1940s by demonstrating that bacterial viruses were inhibited by mustard gas [2]. These findings were later extended to mammalian cells [3]. Berger and colleagues [4] proposed a general hypothesis for the mechanism of cell death caused by alkylating agents: (1) DNA strand breaks occur as a direct result of alkylation; (2) poly(ADP-ribose)

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polymerase (PARP; EC 2.4.2.30) is activated by DNA breaks to synthesize large homopolymers of ADP-ribose, thereby depleting cellular NAD levels; and (3) reduced NAD inhibits glycolysis and causes a loss of the ATP required to maintain normal cellular metabolism. Papirmeister applied this hypothesis to explain the cytotoxicity and vesication caused by HD exposure [5].

Our goal has been to test the Berger/Papirmeister hypothesis for HD-initiated toxicity using in vitro models. HD exposure was shown to lower NAD levels in two systems: isolated, human peripheral blood lymphocytes (PBLs) [6], and human skin grafted to nude mice [7]. The loss of NAD was partially protected by selective inhibitors of PARP [8]. According to Berger's hypothesis, decreased cellular NAD levels should lead to decreased ATP levels. Exposure of human PBLs to HD was indeed found to lower the amounts of extractable ATP and, furthermore, pretreatment with PARP inhibitors conferred some protection against the ATP loss [9]. HD exposure also initiated a concentration-dependent decrease in PBL viability [10]. Pre-treatment of cells with PARP inhibitors, before exposure to HD, preserved viability to an extent that correlated closely ($r = 0.94$) with the IC_{50} values for PARP inhibition in vitro [11].

A systematic survey of cellular polypeptides from PBLs exposed to HD revealed increased solubility of core histones H3 and H2B after HD exposure. The amino-terminus of H2B had been removed, perhaps by a chymotrypsin-like protease [12]. Cells with altered histone solubility also showed extensive DNA fragmentation [12].

The goals of the present study were: (1) to characterize the time and concentration dependence of HD-initiated DNA fragmentation in human PBLs; and (2) to examine whether DNA damage can be mitigated by treatment with the selective PARP inhibitors that have been shown previously to protect against metabolic injury.

2. Materials and methods

2.1. Materials

Reagents for making buffers were purchased from Fisher Scientific Co., Pittsburgh, PA. Garamycin and RPMI 1640 medium with L-glutamine, 25 mM HEPES without sodium bicarbonate (RPMI 1640M) were purchased from Whittaker M.A. Bioproducts, Walkersville, MD. Percoll was purchased from Pharmacia, Piscataway, NJ. Trypan blue, propidium iodide (PI), niacinamide, niacin, 3-aminobenzamide, and reagents for preparing agarose gels were purchased from Sigma, St. Louis, MO. The PARP inhibitors 3,4-dihydro-5-3-(methylamino)-propoxy-1-(2*H*)-isoquinolinone, monohydrochloride (ICD 2250) and 3,4-dihydro-5-methyl-1-(2*H*)-isoquinolinone (ICD 2163) were gifts from Parke Davis. A Beckman 70 pH meter, Becton Dickinson FACSort, and Bio-Rad horizontal gel system were used to determine pH, cellular fluorescence, and DNA patterns respectively. Sulfur mustard and Lewisite were obtained from the Chemical Research, Development, and Engineering Center, Aberdeen Proving Ground, MD and were determined to be $>99\%$ pure by gas chromatography.

2.2. Isolation of human PBLs

Blood (200 ml) was drawn from healthy, young human volunteers (age range 19–46 years, seven males and three females) under an approved human use protocol. PBLs were isolated from blood by centrifugation on a Percoll gradient (density = 1.080 at 20°C) as described previously [13,14]. Cells were washed twice with calcium-, magnesium-free Tyrode's buffer, and once with RPMI containing garamycin. Between washes, the cells were centrifuged at $900 \times g$ for 10 min at 20°C. The number of PBLs obtained was determined by counting with a hematocytometer. Cells were re-suspended in an appropriate volume of RPMI 1640M medium and allocated into tissue culture plates.

2.3. Exposure of cells to HD or Lewisite

Exposure was performed in serum-free RPMI 1640M medium using final concentrations of HD ranging from 10^{-6} to 10^{-3} M, as described [10–12]. To ensure safety from highly toxic HD vapors, all work with HD was conducted in a Class II, Type B2 tissue culture hood (Baker Company, Sanford, ME) fitted with a five train filter system containing two HEPA and three HEGA filters. The cells were main-

tained in a humidified CO_2 incubator at 37°C for the indicated post-exposure time interval. For time course experiments, cells were exposed to 3×10^{-4} M HD at time zero and aliquots of exposed cells were analyzed at 2, 4, 8, 12, 16, 20, or 24 h after exposure.

For comparison, PBLs were exposed to HD (3×10^{-4} M), Lewisite (3×10^{-4} M), or heat (55°C for 30 min) and genomic DNA patterns were compared 24 h later. Lewisite was chosen because it is a vesicating agent, which is more cytotoxic than HD, but it is not known to alkylate DNA.

2.4. HD and PARP inhibitors

Cells were combined with RPMI 1640M medium and PARP inhibitor 15 min before adding the appropriate volume of HD. Each experiment consisted of four groups: (1) PBLs exposed to RPMI 1640M alone; (2) PBLs exposed to RPMI 1640M with HD; (3) PBLs exposed to RPMI 1640M with PARP inhibitor; or (4) PBLs equilibrated for 15 min with RPMI 1640M containing PARP inhibitor and then exposed to HD. DNA was extracted at either 2, 4, 6, 12, 16, 20, or 24 h after HD exposure and analyzed by gel electrophoresis.

In some experiments, PARP inhibitor was added to cell medium at either 1 h before, or at 1, 2, 4, 8, 12, 16, or 24 h after exposure to 3×10^{-4} M HD. Cells were incubated with HD for a total of 24 h before analysis.

2.5. Determination of PBL viability

Cell viability was defined operationally as the ability of PBLs to exclude PI. Freshly isolated PBLs were placed in 200 μl volumes at 1×10^{6} cells/ml for viability experiments. After treatment, cells were removed from each well, incubated 3–5 min at room temperature with 50 μl of PI (50 mg/ml), and assayed for fluorescent PI staining using a Becton Dickinson FACSort and the accompanying Lysis software [11,14]. At least 10 000 cells were assayed for each sample in each analysis.

2.6. DNA extraction and electrophoresis

DNA analysis experiments were done using from 1–1.5$\times10^{7}$ cells/well (24 well tissue culture plates) in a total volume of 1 ml. Cells were transferred to 15 ml conical tubes by rinsing tissue culture plates thrice in physiological saline, pH 7.5. Total genomic DNA was purified by sequential applications of protease K, RNAse, and a differential salt extraction protocol. DNA was precipitated overnight with ethanol at −10°C (Gnome Kit, Bio 101, La Jolla, CA).

Solubilized DNA in TE buffer was evaluated by electrophoresis using 1–3% (w/v) agarose gels as described [12]. The high concentration of agarose (3% w/v) was used to detect oligonucleosome-sized DNA fragments. DNA from approximately 10^{6} cell equiv-

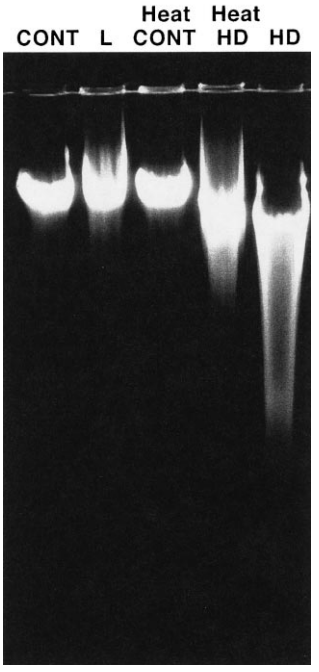

Fig. 1. Electrophoresis (2.5% agarose gel) of total genomic DNA extracted from human PBLs. Each lane contains 1×10^{6} cell equivalents of DNA extracted from cells which were maintained at 37°C in a humidified CO_2 incubator for 24 h after the indicated treatment. Lane 1 contains DNA from control PBLs (CONT); lane 2 contains DNA from PBLs exposed to 3×10^{-4} M Lewisite (L); lane 3 contains DNA from PBLs killed by heating at 55°C for 30 min (Heat CONT); lane 4 contains DNA from human PBLs exposed to 3×10^{-4} M HD and subsequently heated at 55°C for 30 min (Heat HD); and lane 5 contains DNA from human lymphocytes exposed to 3×10^{-4} M HD. The percent viability for cell populations used to obtain DNA for lanes 1–5 was: 98, 33, 59, 60 and 62%, respectively, as measured by flow cytometry using propidium iodide exclusion with a standard deviation of less than 1%.

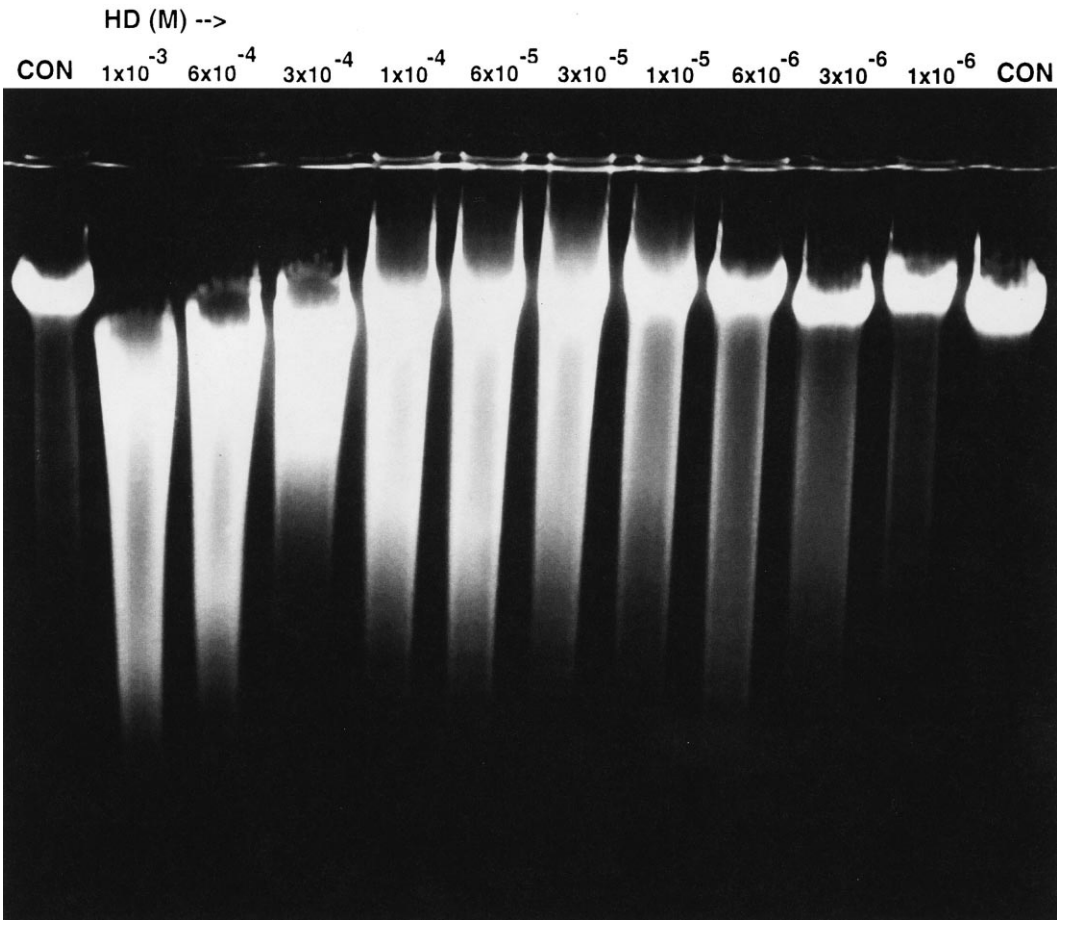

Fig. 2. Effect of increasing HD concentration on DNA from human lymphocytes. Lanes 1 and 12 contain DNA extracted from control PBLs that were exposed only to medium (CON). Lanes 2–11 contain DNA from PBLs of the same donor that were exposed to HD at applied concentrations of: 10^{-3}, 6×10^{-4}, 3×10^{-4}, 10^{-4}, 6×10^{-5}, 3×10^{-5}, 10^{-5}, 6×10^{-6}, 3×10^{-6}, or 10^{-6} M HD, respectively.

alents was loaded into each gel lane and electrophoresis was performed by application of constant voltage (100 V across 10 cm gels) at 10°C.

3. Results

3.1. HD exposure causes DNA fragmentation

DNA fragmentation was defined as: (1) smaller average size of genomic DNA, as judged by the distance that DNA migrated into 2–3% (w/v) agarose gels and (2) qualitative widening of the DNA band, relative to DNA from control cells. Exposure to HD consistently resulted in decreased cell viability and extensive DNA fragmentation, relative to control populations of cells from the same donor (Fig. 1). Heating the PBLs to 55°C for 30 min before exposure to HD (3×10^{-4} M), followed by incubation at 37°C for 24 h, resulted in approximately the same loss of viability (53% cell viability) only slightly altered the HD-initiated DNA fragmentation.

The effects of HD on genomic DNA were not simply due to cytotoxicity. Heating PBLs to 55°C for 30 min, in the absence of HD, resulted in a significant reduction in cell viability at 24 h (59% viable at 24 h), but had no effect on the DNA pattern observed on agarose gels (Fig. 1). Lewisite, a potent vesicating agent, was more cytotoxic (34% of PBLs viable at 24 h) than either HD or heating cells to 55°C. However, genomic DNA extracted from Lewisite-exposed cells was indistinguishable by agarose gel electrophoresis from DNA extracted from control cells.

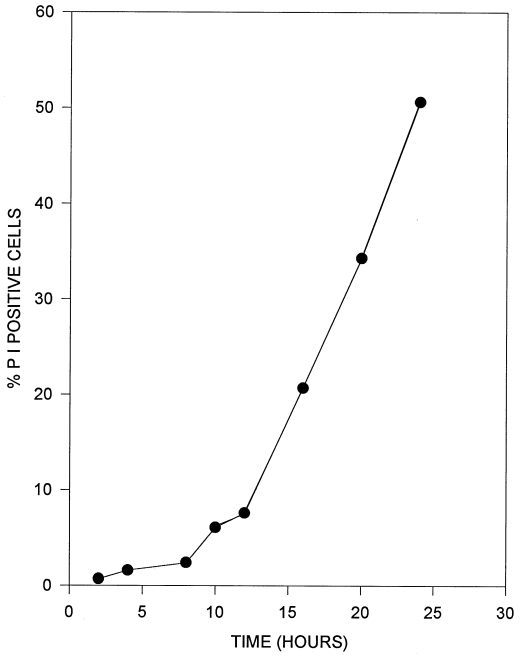

Fig. 3. Post-exposure time dependence of HD-initiated cytotoxicity. The number of damaged cells was assessed by PI incorporation into the cell nucleus. Human PBLs were exposed to 3×10^{-4} M HD at time zero. At the indicated post-exposure times, cells were removed from the 37°C incubator and treated with PI for 3 min at room temperature. For each result shown, 10^4 cells were examined individually on a FACSort to determine the percentage PI-positive cells.

3.2. Effect of HD concentration

DNA fragmentation patterns depended upon the concentration of HD (Fig. 2). Changes in the DNA patterns were observed at 24 h after exposure to HD concentrations as low as 6×10^{-6} M (Fig. 2). As the HD concentration was increased, the average size of extracted DNA fragments apparently decreased, as judged by migration distance of the center of mass of the DNA bands in agarose gels. At the highest HD concentrations used (10^{-3} M), genomic DNA was converted to a heterogeneous population of fragments that migrated farther into 3% agarose gels than did control DNA from the same donor (Fig. 2).

We found gel electrophoresis to be a sensitive and reproducible method for detecting HD-induced fragmentation. Qualitative DNA changes were routinely detected using as few as 10^6 cell equivalents. The assay was sensitive to changes caused by HD concentrations as low as 6×10^{-6} M HD at 24 h after exposure.

3.3. Effect of post-exposure time interval

There was no appreciable loss of viability in the control cells during the entire 24 h period studied. In contrast, the viability of cells exposed to HD was significantly decreased as early as 8 h after exposure, and continued to decline over the 24 h incubation period (Fig. 3). When DNA from control cells was examined on 1.5% agarose gels, there also was no change during the 24 h post-exposure interval. Exposure to 3×10^{-4} M HD, however, caused DNA fragmentation as early as 2 h after exposure (Fig. 4).

DNA fragmentation increased during the first 12 h after HD exposure, manifesting as a thin 'tail' of DNA that migrated progressively farther in agarose gels. The DNA tail consisted of an oligonucleosome-sized 'ladder' of decreasing size. Beginning at 6–16 h

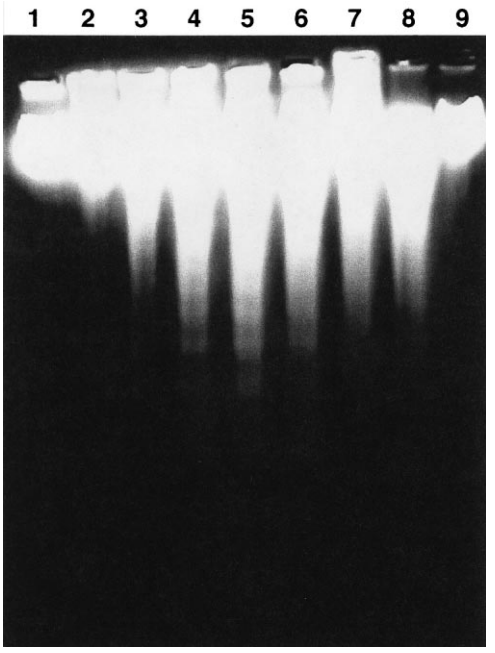

Fig. 4. Post-exposure time dependence of HD-initiated DNA fragmentation. Human PBLs were exposed to 3×10^{-4} M HD. At the indicated times after exposure, cells were removed from the 37°C incubator and the genomic DNA was extracted. Lane 1 contains DNA from control cells at time zero and lane 9 contains DNA from control cells after a 24 h incubation. Lanes 2–8 contain DNA extracted from cells at 2, 4, 6, 12, 16, 20 or 24 h after exposure, respectively.

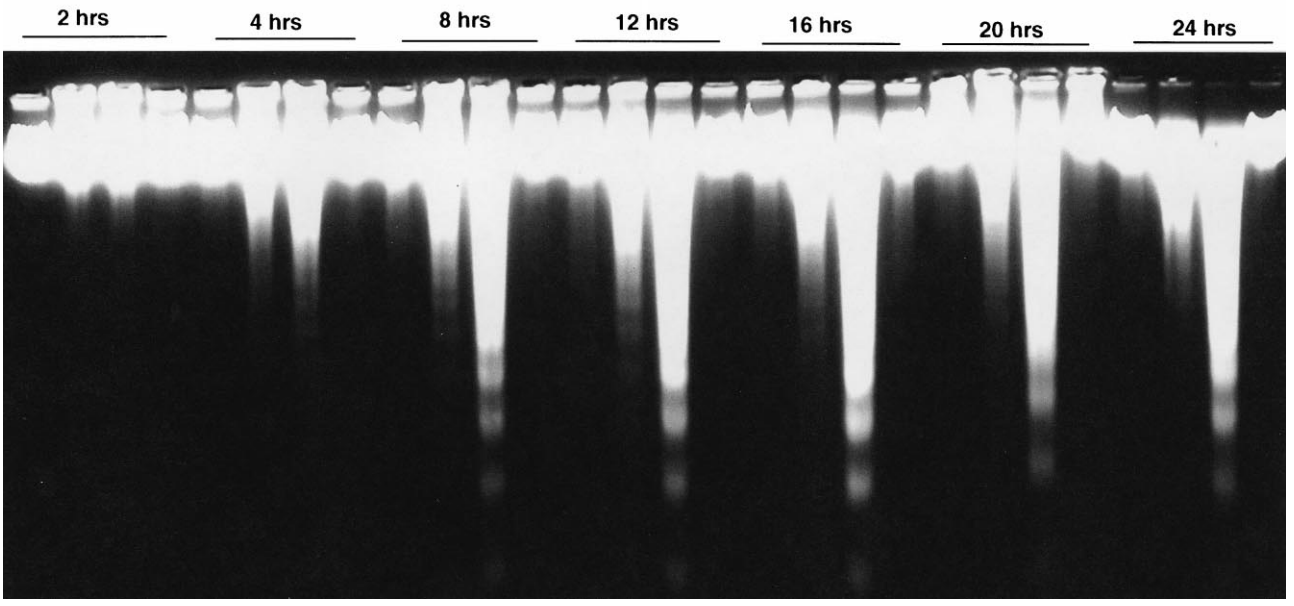

Fig. 5. Effect of PARP inhibitors on genomic DNA fragmentation as a function of the post-exposure time interval. For each time point, given in hours at the top of the gel, there are four lanes that contain DNA from cells treated as follows (left to right): (1) medium alone (controls); (2) 3×10^{-4} M HD; (3) 3×10^{-4} M HD+3×10^{-4} M PARP inhibitor; (4) 3×10^{-4} M PARP inhibitor alone. Beginning at 4 h after HD exposure, the PBL DNA showed detectable fragmentation. Initially, DNA extracted from cells treated with HD alone showed a 'ladder pattern' of discrete, oligonucleosome-sized DNA fragments, characteristic of apoptotic cell death. At approximately 16 h after exposure, the pattern changed qualitatively. The DNA migrated as a heterogeneous, 'broad band' of fragments smaller than DNA extracted from control cells or cells treated only with PARP inhibitors. In contrast, the cells exposed to HD in the presence of a PARP inhibitor retained the 'ladder' configuration over the entire 24 h post-exposure period. Identical results were obtained using ICD 2250 or ICD 2163 as the PARP inhibitor.

after exposure, depending on the applied concentration of HD, the DNA fragmentation pattern changed. The DNA tail, which was lengthening during the first 12 h after exposure, began to decrease in length and the ladder pattern disappeared. By 24 h after HD exposure, the ladder pattern was no longer detectable and the DNA migrated as a heterogeneous band with a center of mass apparently smaller than control DNA from the same donor (Fig. 4).

3.4. Effect of PARP inhibitors on DNA fragmentation

Cells incubated in medium alone, or in medium with only PARP inhibitors, showed a single narrow band of DNA that migrated a similar distance in the agarose gel for all time points studied (Fig. 5). Pretreatment of cells with PARP inhibitors, in contrast, altered the HD-initiated DNA fragmentation pattern. Cells treated with the most potent PARP inhibitors, ICD 2163 or ICD 2250, in combination with HD, consistently showed a pronounced 'tailing' of DNA that included a discrete, oligonucleosome-sized DNA ladder pattern for the entire 24 h post-exposure interval (Fig. 5).

3.5. Effect of PARP inhibitors depended on time of addition

The PARP inhibitors preserved the DNA ladder pattern only if added either 1 h before HD, or during the first 2 h after HD. If inhibitors were added 4 h after HD, the DNA ladder remained detectable, but was superimposed with heterogeneous DNA band. By 8 h after HD exposure, addition of the PARP inhibitors had no detectable effect (Fig. 6). Protection of viability by PARP inhibitor addition in PBLs exposed to HD also decreased as the time of addition was delayed, as reported previously [14].

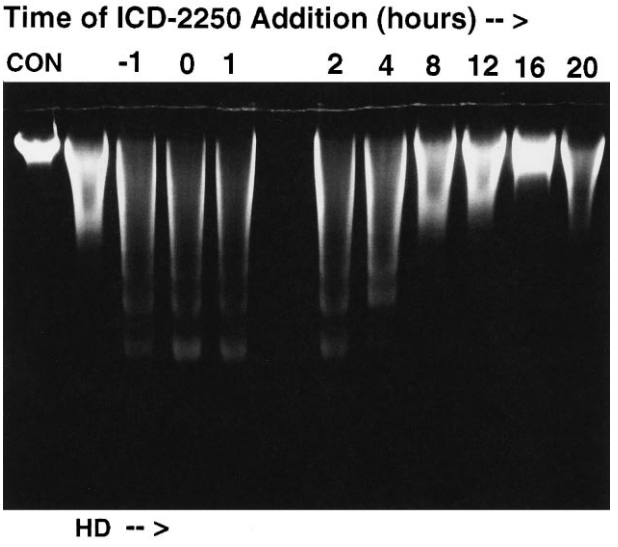

Fig. 6. Maintenance of the 'DNA ladder' depends upon the time of PARP addition. PBLs were exposed to 3×10^{-4} M HD. The PARP inhibitor, ICD 2250, was added at the indicated post-exposure times, and cells were then incubated for a total of 24 h. As shown in Fig. 5, pretreatment (−1 h) or immediate addition (0 h) of the PARP inhibitor after HD exposure resulted in a 'DNA ladder' pattern of fragmentation. However, if the PARP inhibitor was added later than 8 h after HD, it failed to arrest DNA fragmentation at the ladder stage. Identical results were obtained using ICD 2163 under the same conditions.

4. Discussion

4.1. HD exposure causes genomic DNA fragmentation

We previously reported that exposure of human PBL preparations to HD causes time- and concentration-dependent decreases in viability [10], NAD levels [8], and ATP levels [9], as well as qualitative alterations in core histones and genomic DNA [12]. The present study found that fragmentation of nuclear DNA, as judged by gel electrophoresis, also depended upon the HD concentration and the post-exposure time interval.

Changes in gel electrophoresis patterns were neither artifacts of DNA extraction, nor the result of non-specific cytotoxicity, because DNA fragmentation was not observed in PBLs that were killed with heat or Lewisite (Fig. 1). HD-initiated DNA fragmentation was only slightly altered by heating the cells to 55°C for 30 min before exposure (Fig. 1). This suggested that fragmentation was not solely dependent upon the activity of heat-labile DNases. DNA strand breaks probably resulted from direct interaction of the HD on the genome, including alkylation [3] and disruption of the histone-DNA core particle [12].

While studying the effects of HD concentration and the post-exposure time interval, we found evidence for at least two distinct types of DNA fragmentation. There was an initial type of fragmentation caused by HD exposure that resulted in a DNA ladder pattern identical to that reported to occur during apoptotic cell death [15]. With increased time after exposure, however, the ladder pattern disappeared and a wider band of DNA was observed with a tail pattern similar to that seen in necrotic cell death [15]. The conversion of the dominant type of DNA fragmentation from a ladder to the wide band occurred 6–16 h after HD exposure, depending on the applied concentration of HD. DNA fragmentation and time of conversion occurred earlier after exposure to higher HD concentrations. The delayed conversion from apoptotic to necrotic DNA fragmentation was consistent with earlier reports of a concentration-dependent latent period of 6–16 h after HD exposure, during which the cell membrane remains functional and the cells are capable of operating metabolic pathways [10,14,16].

4.2. Mitigation of HD effects by PARP inhibitors

Treatment of PBLs with PARP inhibitors was shown previously to reduce the membrane damage caused by HD exposure [17]. Likewise, the PARP inhibitors could partially block HD-initiated decreases in cell viability [10], NAD levels [6], and ATP levels [9]. The degree of protection was proportional to the ability of the compound to inhibit PARP in vitro [11]. The most potent PARP inhibitors tested, ICD 2163 and 2250, almost completely blocked the HD-induced changes in viability [11,14] and metabolism caused by HD [8,9].

In contrast with metabolic protection, the PARP inhibitors were unable to prevent DNA fragmentation in cells exposed to HD (Fig. 5). The inhibitors were able, however, to alter the fragmentation patterns. Addition of PARP inhibitors to the cell medium blocked the time-dependent conversion of DNA fragmentation from the initially dominant lad-

der pattern to the later dominant wide band DNA pattern (Fig. 5). Thus, it appeared that the PARP inhibitors changed the kinetics of DNA cleavage so that the cell death remained apoptotic during the entire 24 h post-exposure time interval.

The mechanism by which the PARP inhibitors arrest cells at the stage of a DNA ladder is unknown, but it could involve preservation of condensed chromatin. Histones have been linked to pADP-RP by a 'histone shuttle mechanism' of DNA repair [18]. Inhibition of pADP-RP effectively blocks this mechanism in HD-treated PBLs. This would prevent the cell from unwinding the core particle for repair, thereby resulting in the oligonucleosome-sized 'DNA ladder' fragments observed. In contrast, unprotected cells would be expected to show a heterogeneous distribution of smaller fragments because pADP-RP activation removes histones and exposes more DNA for HD alkylation and subsequent strand breakage.

The PARP inhibitors were only effective at preventing the conversion from an apoptotic-like DNA fragmentation pattern to a necrotic-like DNA fragmentation pattern if added within 8 h of HD exposure. This paralleled the time course reported for protection of the HD-initiated decrease in membrane function [10,14]. The PARP inhibitors were able to preserve some metabolic pathways when added in a delayed treatment mode. The relationship between metabolic and nuclear effects of the PARP inhibitors is unknown, but both effects apparently involved cellular events that occur during the first several hours after exposure to a cytotoxic concentration of HD.

4.3. Therapeutic switch from necrosis to apoptosis?

The PARP inhibitors may exert an overall beneficial effect by reducing the normal cellular response to extensive DNA alkylation. As predicted by Berger, inhibition of repair processes that require PARP would be expected to preserve cellular energy levels and, thereby, to slow loss of metabolic function and disruption of the cell membrane [4].

'Knock-out' mice that completely lack PARP activity (PARP−/−) were shown to develop structurally normal skin that forms an intact barrier [19]. However, cells from PARP−/− mice showed impaired proliferation [19] and faulty DNA repair mechanisms [20] when exposed to the stress of DNA damaging agents. Other in vitro or ex vivo model systems have shown that reduction of PARP activity impairs normal cellular responses to DNA damaging agents, perhaps in a cell cycle-dependent manner [21–23]. Our results with HD suggest that impaired proliferation and DNA repair in PARP-deficient cells may result from a greater fraction of damaged cells arrested in a metabolically active but apoptotic state, perhaps because chromatin can not be opened efficiently for DNA repair.

The ability of PARP inhibitors to alter the cytotoxic mechanism of DNA alkylating agents was demonstrated in human cells by studying metabolic and DNA changes induced by the inhibitors [24]. It appeared that SM exposure activated an early apoptotic-like mechanism of cell death. In the absence of PARP inhibitors, this apoptotic mechanism was replaced with a necrotic mechanism at 4–8 h after SM exposure. The presence of inhibitors of PARP in the culture medium either prevented the necrotic mechanism or limited it to a minor role in the death of the cell.

Our initial results with SM-mediated cell death can be compared with the mechanism of HT-29 cell death initiated by H_2O_2, another DNA damaging cytotoxic agent [25]. The H_2O_2-initiated HT-29 cell death was found to occur by an immediate necrotic-type cell death mechanism, followed by a delayed apoptotic mechanism. Although PARP inhibitors were able to block the immediate necrotic pathway, the delayed apoptotic pathway was nevertheless activated. It was concluded that the delayed pathway caused cell death and the eventual detachment of the HT-29 cells from the tissue culture plates [25]. The experiments presented in this article support our conclusion that the initial mechanism of SM cytotoxicity was the activation of an apoptotic-like pathway. However, in contrast with H_2O_2, exposure to SM resulted in a latent necrotic cytotoxicity that was manifest only when metabolic cofactors had been reduced to a level of about 50% of control cells.

The role of PARP in DNA damage-mediated cell death initiated by H_2O_2 in a motor neuron cell line has also been investigated [26]. PARP activation occurred in this in vitro model, and the introduction of PARP inhibitors blocked an increased immunologic

staining of poly(ADP-ribose) polymers. Although the mechanism of cell death was not defined, the authors were able to conclude that significant DNA fragmentation occurred both in the presence and absence of PARP inhibitors. The effectiveness of PARP inhibitors in altering the mechanism of DNA damage-mediated cell death was subsequently demonstrated in whole animal experiments [27].

Using an ischemia-induced cell model in vivo, moderate concentrations of PARP inhibitors were shown to reduce the infarction volume damage following reperfusion in male Wistar rats with experimentally produced middle cerebral artery occlusion [27]. PARP inhibitors effectively prevented or reduced cytotoxicity initiated by DNA damaging agents in both in vitro and in vivo experiments. However, the order of the activation of the different mechanisms of cell death (apoptosis and necrosis) was not resolved. To determine treatment regimens and limiting treatment schedules for tissue exposed to DNA damaging conditions, the kinetics of cell death mechanism(s) should be extensively investigated.

We speculate that the PARP inhibitors may have therapeutic utility at preventing or reducing the lesion initiated by HD exposure if they promote a non-proliferative, apoptotic state in basal keratinocytes of HD-exposed skin. Although PARP inhibition impairs suicide DNA repair function, it does not appear to affect normal functions at the cellular and tissue level. The inhibition of the necrotic suicidal repair mechanism should result in the amelioration of the overall tissue injury. Unlike the necrotic response to HD alone, the PARP inhibitors favor an apoptotic state, which appears to be the normal maturation mechanism of keratinocytes in skin. Thus with the HD-DNA damaged cells in a normal apoptotic state the cells may be passed to the stratum corneum where they are eliminated or these cells may be reabsorbed as occurs in other tissues where apoptotic cell death takes place. In either case, the dying cells would be eliminated instead of rapidly lysing to release pro-inflammatory components and lysosomal proteases as seen in necrotic cell death. Meaningful tests of this hypothesis, as well as evaluation of PARP inhibitors as therapeutic anti-vesicants, await development of suitable animal models for intact human skin.

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References

[1] B.P. McNamara, Medical Aspects of Chemical Warfare, DTIC AD-770 735, U.S. Army Chemical Research and Development Laboratories, Army Chemical Center, MD, 1960.
[2] R.M. Herriott, W.H. Price, J. Gen. Physiol. 32 (1948) 63–68.
[3] M. Fox, D. Scott, Mutat. Res. 75 (1980) 131–168.
[4] N.A. Berger, G.W. Sikorski, S.J. Petzold, K.K. Kurohara, J. Clin. Invest. 63 (1979) 1164–1171.
[5] B. Papirmeister, C.L. Gross, J.P. Petrali, H.L. Meier, J. Toxicol. Cutan. Ocul. Toxicol. 3 (1984) 395–410.
[6] H.L. Meier, C.T. Lusco, C.L. Gross, L.M. Graham, R.C. Krebs, J.B. Johnson, Toxicologist 7 (1987) 168.
[7] C.L. Gross, H.L. Meier, B. Papirmeister, F.B. Brinkley, J.B. Johnson, Toxicol. Appl. Pharmacol. 81 (1985) 85–90.
[8] H.L. Meier, C.L. Gross, B. Papirmeister, Toxicol. Lett. 39 (1987) 109–122.
[9] H.L. Meier, E.T. Clayson, S.A. Kelly, C.M. Corun, In Vitro Toxicol. 9 (1996) 135–139.
[10] H.L. Meier, J.B. Johnson, Toxicol. Appl. Pharmacol. 113 (1992) 234–239.
[11] E.T. Clayson, S.A. Kelly, H.L. Meier, Cell Biol. Toxicol. 9 (1993) 165–175.
[12] C.B. Millard, H.L. Meier, C.A. Broomfield, Biochim. Biophys. Acta 1224 (1994) 389–394.
[13] A. Boyum, Scand. J. Clin. Lab. 21 (1968) 77–89.
[14] H.L. Meier, Cell Biol. Toxicol. 12 (1996) 147–153.
[15] S.A. Altman, L. Randers, G. Rao, Biotech. Prog. 9 (1993) 671–674.
[16] B. Papirmeister, A.J. Feuster, S.I. Rovinson, R.D. Ford, In: Mustard Gas, CRC Press, Boca Raton, FL, 1991, pp. 299–348.
[17] J.P. Petrali, S.B. Oglesby, H.L. Meier, Ultrastruct. Pathol. 14 (1988) 253–262.
[18] F.R. Althaus, L. Hofferer, H.E. Kleczkowska, M. Malanga, H. Naegeli, P.L. Panzeter, C.A. Realini, F.R. Althaus, Mol. Cell. Biochem. 138 (1994) 53–59.
[19] Z. Wang, B. Auer, L. Stingl, H. Berghammer, C. Haidacher, M. Schweiger, E.F. Wagner, Genes Dev. 9 (1995) 509–520.
[20] J.M. de Murcia, C. Niedergang, C. Trucco, M. Ricoul, B. Dutrillaux, M. Mark, F.J. Oliver, M. Masson, A. Dierich, M. LeMeur, C. Walztinger, P. Chambon, G. de Murcia, Proc. Natl. Acad. Sci. USA 94 (1997) 7303–7307.
[21] V. Schreiber, D. Hunting, C. Trucco, B. Gowans, D. Grunwald, G. De Murcia, J.M. De Murcia, Proc. Natl. Acad. Sci. USA 92 (1995) 4753–4757.
[22] D.S. Rosenthal, C.M.G. Simbulan, M.E. Smulson, Biochimie 77 (1995) 439–443.

[23] S. Chatterjee, N.A. Berger, Mol. Cell. Biochem. 138 (1994) 61–69.
[24] H.L. Meier, C.B. Millard, In Vitro Cell. Dev. Biol. 31 (1995) 47A.
[25] A.J.M. Watson, J.N. Askew, R.S.P. Benson, Gastroenterology 109 (1995) 472–482.
[26] M.R. Cookson, P.G. Ince, P.J. Shaw, J. Neurochem. 70 (1998) 501–508.
[27] A.Y. Sun, J.S. Cheng, Acta Pharmacol. Sin. 19 (1998) 104–108.